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AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions, and listings, of claims in this application.

1. (Previously Presented) A process for producing transglutaminase having an enzymatic activity comprising:

(a) incubating a denatured transglutaminase in an acidic aqueous medium;

(b) diluting the denatured transglutaminase in the acidic aqueous medium by about 5-fold to about 400-fold; and

(c) adjusting the pH of said aqueous medium to a neutral pH by adding an alkali to said aqueous medium.

2. (Original) The process as claimed in claim 1, wherein the aqueous medium further comprises a reducing agent.

3. (Original) The process as claimed in claim 2, wherein the reducing agent is selected from the group consisting of dithiothreitol, 2-mercaptoethanol, and tris-(2-carboxyethyl)phosphine.

4. (Original) The process as claimed in claim 1, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.

5. (Original) The process as claimed in claim 4, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

6. (Original) The process as claimed in claim 4, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

7. (Original) The process as claimed in claim 1, wherein the aqueous medium in step (a) further comprises a protein denaturant.

8. (Original) The process as claimed in claim 7, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

9. (Currently Amended) The process as claimed in claim 7, wherein the transglutaminase concentration is ~~from~~ at least 40 mg/ml and the protein denaturant concentration is from 4 to 10 M.

10. (Original) The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 2 to 7.

11. (Original) The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 3 to 5.

12. (Original) The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 3.5 to 4.5.

13. (Previously Presented) The process as claimed in claim 1, wherein said denatured transglutaminase is diluted at least 5-fold.

14. (Previously Presented) The process as claimed in claim 1, wherein said denatured transglutaminase is diluted at least 10-fold.

15. (Previously Presented) The process as claimed in claim 1, wherein said denatured transglutaminase is diluted at least 50-fold.

16. (Original) The process as claimed in claim 1, wherein said incubation is performed at not more than 15°C.

17. (Original) The process as claimed in claim 1, wherein said incubation is performed from 3 to 10°C.

18. (Currently Amended) The process as claimed in claim 1, wherein ~~preceding~~ after said diluting in step (b) ~~, the acidic aqueous medium of~~ said denatured transglutaminase is ~~diluted to~~ at a concentration of not more than 10 mg/ml.

19. (Original) The process as claimed in claim 1, wherein said neutral pH is from 5.8 to 8.5.

20. (Original) The process as claimed in claim 1, wherein said neutral pH is from 6 to 7.

21. (Currently Amended) The process as claimed in claim 1, wherein in step ~~(b)~~(c), the aqueous medium further comprises an accelerator for forming a higher-order native-state transglutaminase structure having enzymatic activity.

22. (Original) The process as claimed in claim 21, wherein the accelerator is selected from the group consisting of an inorganic salt, an organic salt, an amino acid salt, a polyol, an organic solvent, and a surfactant.

23. (Previously Presented) The process as claimed in claim 22, wherein the accelerator is an inorganic salt accelerator, which is selected from the group consisting of calcium chloride and strontium chloride.

24. (Previously Presented) The process as claimed in claim 23, wherein the inorganic salt accelerator concentration is from 0.01 to 10 mM.

25. (Previously Presented) The process as claimed in claim 22, wherein the accelerator is an organic salt accelerator, which is selected from the group consisting of sodium acetate and sodium propionate.

26. (Previously Presented) The process as claimed in claim 25, wherein the organic salt accelerator concentration is from 0.1 to 2 M.

27. (Previously Presented) The process as claimed in claim 22, wherein the accelerator is an amino acid salt accelerator and is arginine hydrochloride.

28. (Previously Presented) The process as claimed in claim 27, wherein the amino acid salt accelerator concentration is from 0.1 to 2 M.

29. (Previously Presented) The process as claimed in claim 22, wherein the accelerator is a polyol accelerator and is polyethylene glycol.

30. (Previously Presented) The process as claimed in claim 29, wherein the polyol accelerator concentration is from 1 to 10%.

31. (Previously Presented) The process as claimed in claim 22, wherein the accelerator is an organic solvent accelerator which is selected from the group consisting of DMSO and DMF.

32. (Previously Presented) The process as claimed in claim 31, wherein the organic solvent accelerator concentration is from 10 to 40%.

33. (Previously Presented) The process as claimed in claim 22, wherein the accelerator is a surfactant and is CHAPS.

34. (Currently Amended) The process as claimed in claim ~~21~~ 33, wherein the surfactant concentration is from 1 to 50 mM.

35. (Currently Amended) The process as claimed in claim 1, further comprising:

~~(c)~~ (d) centrifugating the aqueous medium of (c).

36. (Currently Amended) An isolated transglutaminase obtained by the process of claim 1, which has ~~an~~ a structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

37. (Previously Presented) The process as claimed in claim 1, wherein step (c) further comprises incubating the aqueous medium for more than 1.5 hours subsequent to adjusting the pH to a neutral region.

38. (Currently Amended) A process for producing transglutaminase having an enzymatic activity, which comprises subjecting denatured transglutaminase to the following steps (a) and (b):

(a) a step for forming an intermediate transglutaminase structure; and

(b) a step for forming a higher-order native-state structure exhibiting substantially the same enzymatic activity as native transglutaminase.

39. – 40. (Cancelled)

41. (Original) The process as claimed in claim 38, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.

42. (Original) The process as claimed in claim 41, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

43. (Original) The process as claimed in claim 41, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

44. – 71. (Cancelled)

72. (Original) The process as claimed in claim 38, further comprising:

(c) a step for separating inactive enzyme(s) as aggregate(s) by centrifugation.

73. (Currently Amended) An isolated transglutaminase obtained by the process of claim 38, which has ~~an~~ a structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

74. (Cancelled).

75. (Original) A transglutaminase comprising the following properties (a) to (d):

(a) specific activity of 15 to 25 U/mg provided through measurement of transglutaminase activity by the hydroxamate method;

(b) a molecular ellipticity which is 30 to 70% of that of the native state in a CD spectrum of a near ultraviolet region;

(c) a molecular weight of 36,000 to 40,000 as measured by SDS-polyacrylamide gel electrophoresis; and

(d) lower mobility than that of a native state in native-polyacrylamide gel electrophoresis with a His-Mes buffer system of pH 6.1.

76. (Previously Presented) A food comprising the transglutaminase of Claim 36.

77. (Previously Presented) The food of Claim 76, which is a jelly, yogurt, cheese or meat.

78. (Currently Amended) A toiletry comprising the transglutaminase of Claim ~~26~~ 36.

79. (Previously Presented) A food comprising the transglutaminase of Claim 73.

80. (Previously Presented) The food of Claim 79, which is a jelly, yogurt, cheese or meat.

81. (Previously Presented) A toiletry comprising the transglutaminase of Claim 73.

82. (Previously Presented) A food comprising the transglutaminase of Claim 75.

83. (Previously Presented) The food of Claim 82, which is a jelly, yogurt, cheese or meat.

84. (Previously Presented) A toiletry comprising the transglutaminase of Claim 75.

85. (Previously Presented) In a method of producing a food comprising a transglutaminase, the improvement comprising producing the transglutaminase according to the process of Claim 1.

86. (Previously Presented) In a method of producing a food comprising a transglutaminase, the improvement comprising producing the transglutaminase according to the process of Claim 38.

SUPPORT FOR THE AMENDMENTS

Claims 18, 34-36, 38, 73, and 78 have been amended.

Support for the amendment to Claim 18 is found in the specification on page 7, lines 13-21. Support for Claims 34-36, 73, and 78 is found in the specification as originally filed an in the corresponding claims as originally filed, as well as on page 1, lines 17-19 of the specification. Support for the amendment of Claim 38 is provided by page 13, lines 15-19.

No new matter is added by these amendments.